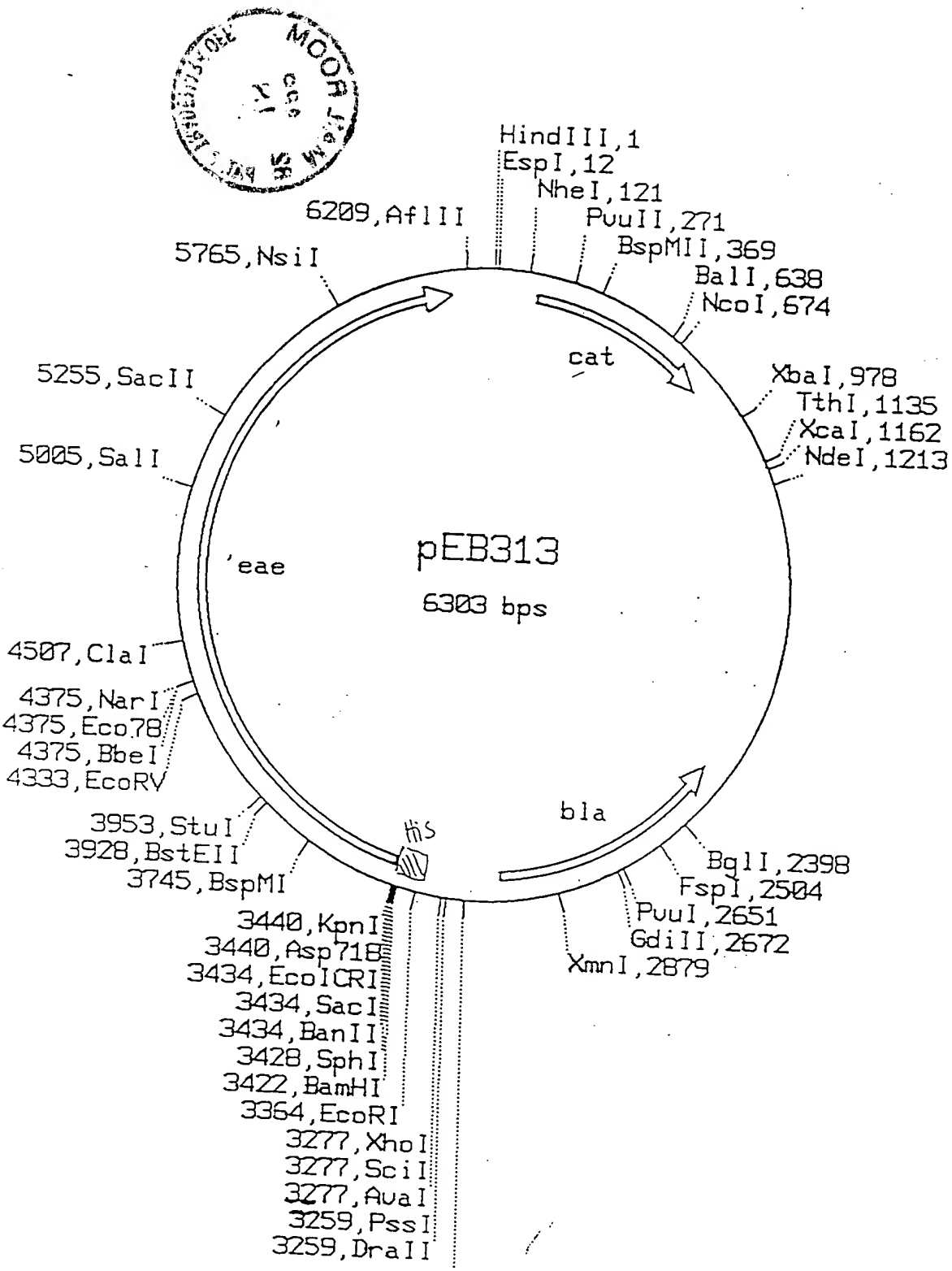
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EspI,12
NheI,121
PuuII,271
BspMII,369
BalI,638
NcoI,674
XbaI,978
TthI,1135
XcaI,1162
NdeI,1213
Bgl I,2398
FspI,2504
PuuI,2651
GdiII,2672
XmnI,2879
3440,KpnI
3440,Asp718
3434,EcoICRI
3434,SacI
3434,BanII
3428,SphI
3422,BamHI
3364,EcoRI
3277,XhoI
3277,SciI
3277,AvaI
3259,PssI
3259,DraII
3953,StuI
3928,BstEII
3745,BspMI
4507,ClaI
4375,NarI
4375,Eco78
4375,BbeI
4333,EcoRV
5005,SalI
5255,SacII
5765,NsiI
6209,AflII
cat
bla
his
'eae
pEB313
6303 bps

FIGURE 1

1	MITHGCYTRT	RHKHKLKKTL	IMLSAGLGLF	FYVNQNSFAN	GENYFKLGSD
51	SKLLTHDSYQ	NRLFYTLKTG	ETVADLSKSQ	DINLSTIWSL	NKHLYSSESE
101	MMKAAPGQQI	ILPLKKLPFE	YSALPLLGSA	PLVAAGGVAG	HTNKLTKMSP
151	DVTKSNMTDD	KALNYAAQQA	ASLGSQLQSR	SLNGDYAKDT	ALGIAGNQAS
201	SQLQAWLQHY	GTAEVNLQSG	DNFDGSSLDF	LLPFYDSEKM	LAFGQVGARY
251	IDSRFTANLG	AGQRFFLPAN	MLGYNVFIDQ	DFSGDNTRLG	IGGEYWRDYF
301	KSSVNGYFRM	RRWHESYHKK	DYDERPANGF	DIRFNGYLPS	YPALGAKLIY
351	EQYYGDNVAL	FNSDKLQSNP	GAATVGVNYT	PIPLVTMGID	YRHGTGNEND
401	LLYSMQFRYQ	FDKSWSQQIE	PQYVNELRTL	SGSRYDLVQR	NNNIILEYKK
451	QDILSLNIPH	DINGTEHSTQ	KIQLIVKSKY	GLDRIVWDDS	ALRSQGGQIQ
501	HSGSQSAQDY	QAILPAYVQG	GSNIYKVTAR	AYDRNGNSSN	NVQLTITVLS
551	NGQVVDQVGV	TDFTADKTSA	KADNADTITY	TATVKKNGVA	QANVPVSFNI
601	VSGTATLGAN	SAKTDANGKA	TVTLKSSTPG	QVVVSAKTAE	MSSALNASAV
651	IFFDQTKASI	TEIKADKTTA	VANGKDAIKY	TVKVMKNGQP	VNNQSVTFST
701	NFGMFNGKSQ	TQATTGNDGR	ATITLTSSSA	GKATVSATVS	DGAEVKATEV
751	TFFDELKIDN	KVDIIGNNVR	GELPNIWLQY	GQFKLKASGG	DGTYSWYSEN
801	TSIATVDASG	KVTLNGKGSV	VIKATSGDKQ	TVSYTIKAPS	YMIKVDKQAY
851	YADAMSICKN	LLPSTQTVLS	DIYDSWGAAN	KYSHYSSMNS	ITAWIKQTSS
901	EQRSGVSSTY	NLITQNPLPG	VNVNTPNVYA	VCVE	

FIGURE 2

```
   1  TCGAGAATGA AATAGAAGTC GTTGTTAAGT CAATGGAAAA CCTGTATTTG GTATTACATA
  61  ATCAGGGAAT AACATTAGAA AACGAACATA TGAAAATAGA GGAAATCAGT TCAAGCGACA
 121  ATAAACATTA TTACGCCGGA AGATAAAATC CGATCTATTA ATATAATTTA TTTCTCATTC
 181  TAACTCATTG TGGTGGAGCC ATAACATGAT TACTCATGGT TGTTATACCC GGACCCGGCA
 241  CAAGCATAAG CTAAAAAAAA CATTGATTAT GCTTAGTGCT GGTTTAGGAT TGTTTTTTTA
 301  TGTTAATCAG AATTCATTTG CAAATGGTGA AAATTATTTT AAATTGGGTT CGGATTCAAA
 361  ACTGTTAACT CATGATAGCT ATCAGAATCG CCTTTTTTAT ACGTTGAAAA CTGGTGAAAC
 421  TGTTGCCGAT CTTTCTAAAT CGCAAGATAT TAATTTATCG ACGATTTGGT CGTTGAATAA
 481  GCATTTATAC AGTTCTGAAA GCGAAATGAT GAAGGCCGCG CCTGGTCAGC AGATCATTTT
 541  GCCACTCAAA AAACTTCCCT TTGAATACAG TGCACTACCA CTTTTAGGTT CGGCACCTCT
 601  TGTTGCTGCA GGTGGTGTTG CTGGTCACAC GAATAAACTG ACTAAAATGT CCCCGGACGT
 661  GACCAAAAGC AACATGACCG ATGACAAGGC ATTAAATTAT GCGGCACAAC AGGCGGCGAG
 721  TCTCGGTAGC CAGCTTCAGT CGCGATCTCT GAACGGCGAT TACGCGAAAG ATACCGCTCT
 781  TGGTATCGCT GGTAACCAGG CTTCGTCACA GTTGCAGGCC TGGTTACAAC ATTATGGAAC
 841  GGCAGAGGTT AATCTGCAGA GTGGTAATAA CTTTGACGGT AGTTCACTGG ACTTCTTATT
 901  ACCGTTCTAT GATTCCGAAA AAATGCTGGC ATTTGGTCAG GTCGGAGCGC GTTACATTGA
 961  CTCCCGCTTT ACGGCAAATT TAGGTGCGGG TCAGCGTTTT TTCCTTCCTG CAAACATGTT
1021  GGGCTATAAC GTCTTCATTG ATCAGGATTT TTCTGGTGAT AATACCCGTT TAGGTATTGG
1081  TGGCGAATAC TGGCGAGACT ATTTCAAAAG TAGCGTTAAC GGCTATTTCC GCATGAGCGG
1141  CTGGCATGAG TCATACAATA AGAAAGACTA TGATGAGCGC CCAGCAAATG GCTTCGATAT
1201  CCGTTTTAAT GGCTATCTAC CGTCATATCC GGCATTAGGC GCCAAGCTGA TATATGAGCA
1261  GTATTATGGT GATAATGTTG CTTTGTTTAA TTCTGATAAG CTGCAGTCGA ATCCTGGTGC
1321  GGCGACCGTT GGTGTAAACT ATACTCCGAT TCCTCTGGTG ACGATGGGGA TCGATTACCG
1381  TCATGGTACG GGTAATGAAA ATGATCTCCT TTACTCAATG CAGTTCCGTT ATCAGTTTGA
1441  TAAATCGTGG TCTCAGCAAA TTGAACCACA GTATGTTAAC GAGTTAAGAA CATTATCAGG
1501  CAGCCGTTAC GATCTGGTTC AGCGTAATAA CAATATTATT CTGGAGTACA AGAAGCAGGA
1561  TATTCTTTCT CTGAATATTC CGCATGATAT TAATGGTACT GAACACAGTA CGCAGAAGAT
1621  TCAGTTGATC GTTAAGAGCA AATACGGTCT GGATCGTATC GTCTGGGATG ATAGTGCATT
1681  ACGCAGTCAG GGCGGTCAGA TTCAGCATAG CGGAAGCCAA AGCGCACAAG ACTACCAGGC
1741  TATTTTGCCT GCTTATGTGC AAGGTGGCAG CAATATTTAT AAAGTGACGG CTCGCGCCTA
1801  TGACCGTAAT GGCAATAGCT CTAACAATGT ACAGCTTACT ATTACCGTTC TGTCGAATGG
1861  TCAAGTTGTC GACCAGGTTG GGGTAACGGA CTTTACGGCG GATAAGACTT CGGCTAAAGC
1921  GGATAACGCC GATACCATTA CTTATACCGC GACGGTGAAA AAGAATGGGG TAGCTCAGGC
1981  TAATGTCCCT GTTTCATTTA ATATTGTTTC AGGAACTGCA ACTCTTGGGG CAAATAGTGC
2041  CAAAACGGAT GCTAACGGTA AGGCAACCGT AACGTTGAAG TCGAGTACGC CAGGACAGGT
2101  CGTCGTGTCT GCTAAAACCG CGGAGATGAC TTCAGCACTT AATGCCAGTG CGGTTATATT
2161  TTTTGATCAA ACCAAGGCCA GCATTACTGA GATTAAGGCT GATAAGACAA CTGCAGTAGC
2221  AAATGGTAAG GATGCTATTA AATATACTGT AAAAGTTATG AAAAACGGTC AGCCAGTTAA
2281  TAATCAATCC GTTACATTCT CAACAAACTT TGGGATGTTC AACGGTAAGT CTCAAACGCA
2341  AGCAACCACG GGAAATGATG GTCGTGCGAC GATAACACTA ACTTCCAGTT CCGCCGGTAA
2401  AGCGACTGTT AGTGCGACAG TCAGTGATGG GGCTGAGGTT AAAGCGACTG AGGTCACTTT
2461  TTTTGATGAA CTGAAAATTG ACAACAAGGT TGATATTATT GGTAACAATG TCAAGAGGTC
2521  GATGTTGCCT AATATTTGGC TGCAATATGG TCAGTTTAAA CTGAAAGCAA GCGGTGGTGA
2581  TGGTACATAT TCATGGTATT CAGAAAATAC CAGTATCGCG ACTGTCGATG CATCAGGGAA
2641  AGTCACTTTG AATGGTAAAG GCAGTGTCGT AATTAAAGCC ACATCTGGTG ATAAGCAAAC
2701  AGTAAGTTAC ACTATAAAAG CACCGTCGTA TATGATAAAA GTGGATAAGC AAGCCTATTA
2761  TGCTGATGCT ATGTCCATTT GCAAAAATTT ATTACCATCC ACACAGACGG TATTGTCAGA
2821  TATTTATGAC TCATGGGGGG CTGCAAATAA ATATAGCCAT TATAGTTCTA TGAACTCAAT
2881  AACTGCTTGG ATTAAACAGA CATCTAGTGA GCAGCGTTCT GGAGTATCAA GCACTTATAA
2941  CCTAATAACA CAAAACCCTC TTCCTGGGGT TAATGTTAAT ACTCCAAATG TCTATGCGGT
3001  TTGTGTAGAA TAATTCCATA ACCACCCCGG CTAAAATATG TATTGTTTTA GTCGGGGCAT
3061  AATTATTTCT TCTTAAGAAA TAACCCTCTT ATAATCAAAT CTACTACTGG TCTTTTTATC
3121  TGCTTAATAG G
```

FIGURE 3

1 GGAAAGATAA ATCCGATCTA TTAATATAAT TTATTTCTCA TTCTAACTCA TTGTGGTGGA
61 GCCATAACAT GAGTACTCAT GGTTGTTATA CCCGGACCCG GCACAAGCAT AAGCTAAAAA
121 AAACATTGAT TATGCTTAGT GCTGGTTTAG GATTGTTTTT TTATGTTAAT CAGAATTCAT
181 TTGCAAATGG TGAAAATTAT TTTAAATTGG GTTCGGATTC AAAACTGTTA ACTCATGATA
241 GCTATCAGAA TCGCCTTTTT TATACGTTGA AAACTGGTGA AACTGTTGCC GATCTTTCTA
301 AATCGCAAGA TATTAATTTA TCGACGATTT GGTCGTTGAA TAAGCATTTA TACAGTTCTG
361 AAAGCGAAAT GATGAAGGCC GCGCCTGGTC AGCAGATCAT TTTGCCACTC AAAAAACTTC
421 CCTTTGAATA CAGTGCACTA CCACTTTTAG GTTCGGCACC TCTTGTTGCT GCAGGTGGTG
481 TTGCTGGTCA CACGAATAAA CTGACTAAAA TGTCCCCGGA CGTGACCAAA AGCAACATGA
541 CCGATGACAA GGCATTAAAT TATGCGGCAC AACAGGCGGC GAGTCTCGGT AGCCAGCTTC
601 AGTCGCGATC TCTGAACGGC GATTACGCGA AAGATACCGC TCTTGGTATC GCTGGTAACC
661 AGGCTTCGTC ACAGTTGCAG GCCTGGTTAC AACATTATGG AACGGCAGAG GTTAATCTGC
721 AGAGTGGTGA TAACTTTGAC GGTAGTTCAC TGGACTTCTT ATTACCGTTC TATGATTCCG
781 AAAAAATGCT GGCATTTGGT CAGGTCGGAG CGCGTTACAT TGACTCCCGC TTTACGGCAA
841 ATTTAGGTGC GGGTCAGCGT TTTTTCCTTC CTGCAAACAT GTTGGGCTAT AACGTCTTCA
901 TTGATCAGGA TTTTTCTGGT GATAATACCC GTTTAGGTAT TGGTGGCGAA TACTGGCGAG
961 ACTATTTCAA AAGTAGCGTT AACGGCTATT TCCGCATGAG GCGCTGGCAT GAGTCATACC
1021 ATAAGAAAGA CTATGATGAG CGCCCAGCAA ATGGCTTCGA TATCCGTTTT AATGGCTATC
1081 TACCGTCATA TCCGGCATTA GGCGCCAAGC TGATATATGA GCAGTATTAT GGTGATAATG
1141 TTGCTTTGTT TAATTCTGAT AAGCTGCAGT CGAATCCTGG TGCGGCGACC GTTGGTGTAA
1201 ACTATACTCC GATTCCTCTG GTGACGATGG GGATCGATTA CCGTCATGGT ACGGGTAATG
1261 AAAATGATCT CCTTTACTCA ATGCAGTTCC GTTATCAGTT TGATAAATCG TGGTCTCAGC
1321 AAATTGAACC ACAGTATGTT AACGAGTTAA GAACATTATC AGGCAGCCGT TACGATCTGG
1381 TTCAGCGTAA TAACAATATT ATTCTGGAGT ACAAGAAGCA GGATATTCTT TCTCTGAATA
1441 TTCCGCATGA TATTAATGGT ACTGAACACA GTACGCAGAA GATTCAGTTG ATCGTTAAGA
1501 GCAAATACGG TCTGGATCGT ATCGTCTGGG ATGATAGTGC ATTACGCAGT CAGGGCGGTC
1561 AGATTCAGCA TAGCGGAAGC CAAAGCGCAC AAGACTACCA GGCTATTTTG CCTGCTTATG
1621 TGCAAGGTGG CAGCAATATT TATAAAGTGA CGGCTCGCGC CTATGACCGT AATGGCAATA
1681 GCTCTAACAA TGTACAGCTT ACTATTACCG TTCTGTCGAA TGGTCAAGTT GTCGACCAGG
1741 TTGGGGTAAC GGACTTTACG GCGGATAAGA CTTCGGCTAA AGCGGATAAC GCCGATACCA
1801 TTACTTATAC CGCGACGGTG AAAAAGAATG GGGTAGCTCA GGCTAATGTC CCTGTTTCAT
1861 TTAATATTGT TTCAGGAACT GCAACTCTTG GGGCAAATAG TGCCAAAACG GATGCTAACG
1921 GTAAGGCAAC CGTAACGTTG AAGTCGAGTA CGCCAGGACA GGTCGTCGTG TCTGCTAAAA
1981 CCGCGGAGAT GAGTTCAGCA CTTAATGCCA GTGCGGTTAT ATTTTTTGAT CAAACCAAGG
2041 CCAGCATTAC TGAGATTAAG GCTGATAAGA CAACTGCAGT AGCAAATGGT AAGGATGCTA
2101 TTAAATATAC TGTAAAAGTT ATGAAAAACG GTCAGCCAGT TAATAATCAA TCCGTTACAT
2161 TCTCAACAAA CTTTGGGATG TTCAACGGTA AGTCTCAAAC GCAAGCAACC ACGGGAAATG
2221 ATGGTCGTGC GACGATAACA CTAACTTCCA GTTCCGCCGG TAAAGCGACT GTTAGTGCGA
2281 CAGTCAGTGA TGGGGCTGAG GTTAAAGCGA CTGAGGTCAC TTTTTTTGAT GAACTGAAAA
2341 TTGACAACAA GGTTGATATT ATTGGTAACA ATGTCAGAGG CGAGTTGCCT AATATTTGGC
2401 TGCAATATGG TCAGTTTAAA CTGAAAGCAA GCGGTGGTGA TGGTACATAT TCATGGTATT
2461 CAGAAAATAC CAGTATCGCG ACTGTCGATG CATCAGGGAA AGTCACTTTG AATGGTAAAG
2521 GCAGTGTCGT AATTAAAGCC ACATCTGGTG ATAAGCAAAC AGTAAGTTAC ACTATAAAAG
2581 CACCGTCGTA TATGATAAAA GTGGATAAGC AAGCCTATTA TGCTGATGCT ATGTCCATTT
2641 GCAAAAATTT ATTACCATCC ACACAGACGG TATTGTCAGA TATTTATGAC TCATGGGGGG
2701 CTGCAAATAA ATATAGCCAT TATAGTTCTA TGAACTCAAT AACTGCTTGG ATTAAACAGA
2761 CATCTAGTGA GCAGCGTTCT GGAGTATCAA GCACTTATAA CCTAATAACA CAAAACCCTC
2821 TTCCTGGGGT TAATGTTAAT ACTCCAAATG TCTATGCGGT TTGTGTAGAA TAATTCCATA
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2941 TAACCTCTTA TAATCAAATC TACTACTGGT CTTTTTATCT GCTTAATAGG TCTCTTTCAA
3001 AGAGACACAT TCACGTTTTC TAGAGTAGGT TGATCCAACC ACGCTGTATA CCAAAGCTGA
3061 ATCACATCAA GCAACAACTA TGCTCACAAC ATCCACACAA TAAAAA

FIGURE 4

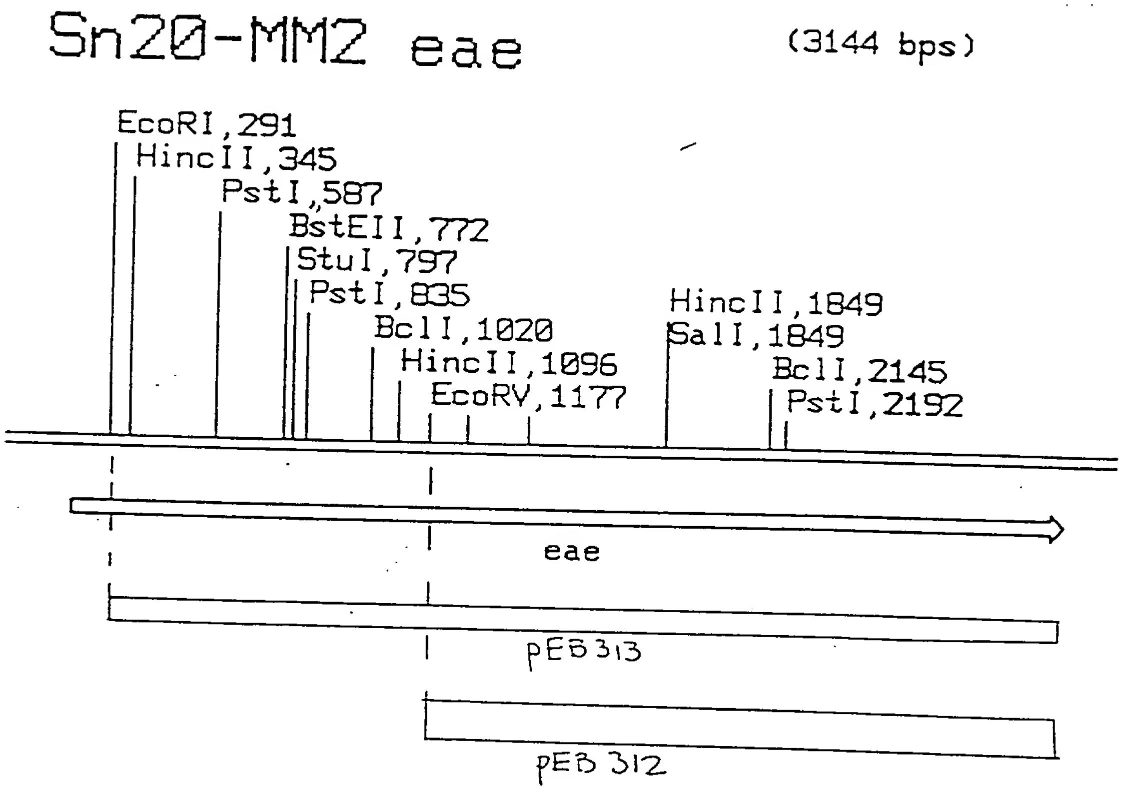
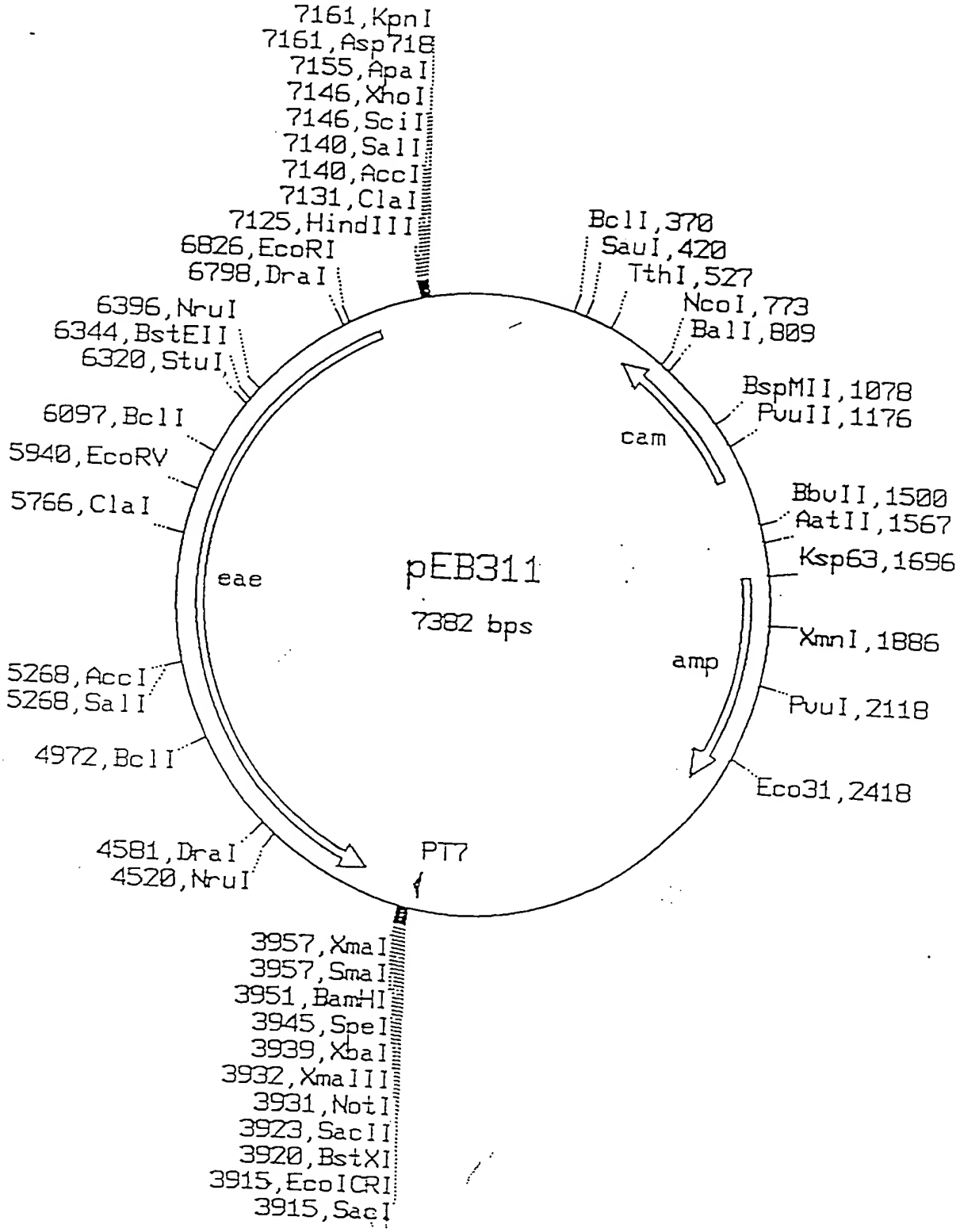


FIGURE 5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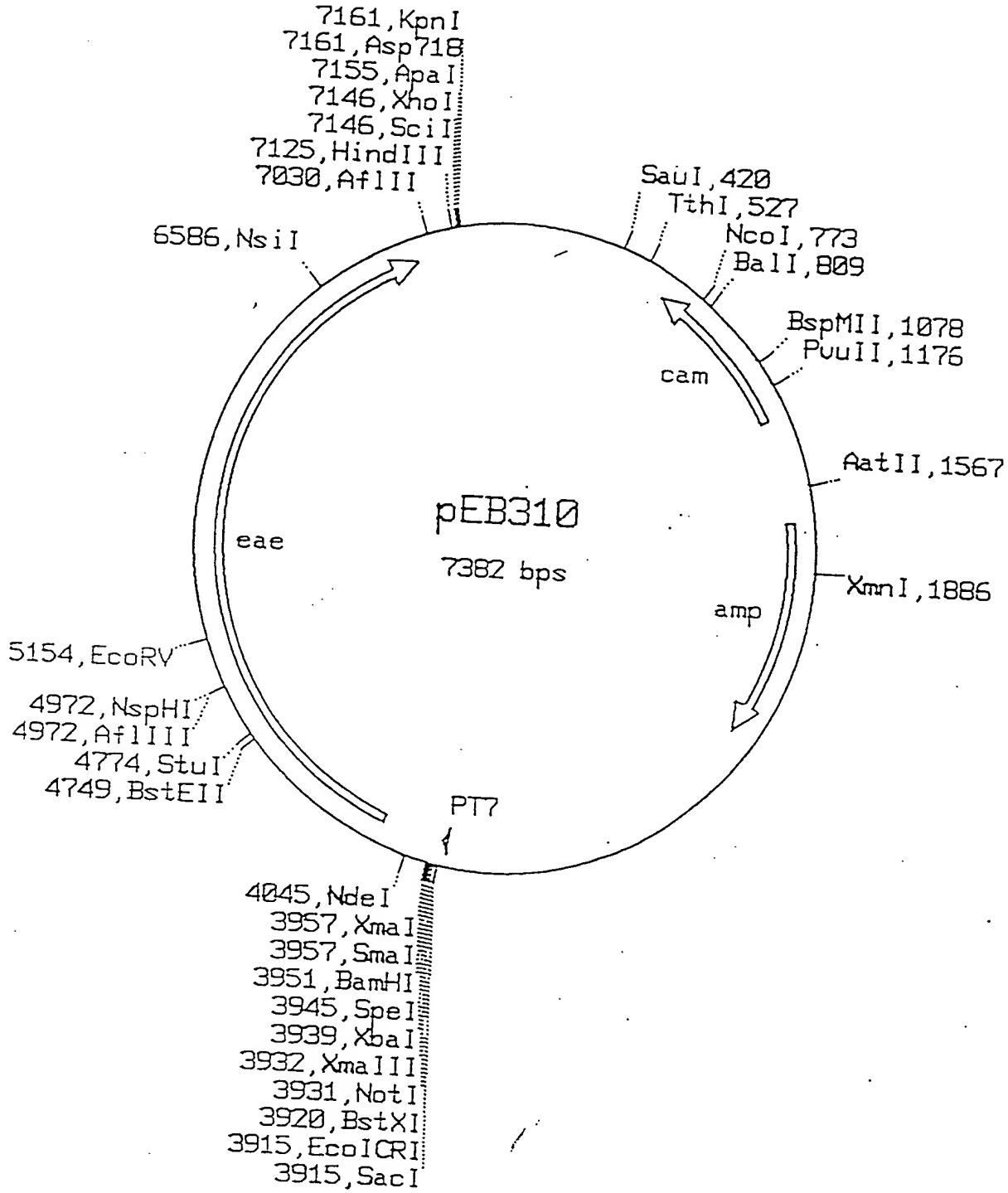
7161,Asp718
7155,ApaI
7146,XhoI
7146,SciI
7140,SalI
7140,AccI
7131,ClaI
7125,HindIII
6826,EcoRI
6798,DraI
6396,NruI
6344,BstEII
6320,StuI
6097,BclI
5940,EcoRV
5766,ClaI
5268,AccI
5268,SalI
4972,BclI
4581,DraI
4520,NruI
3957,XmaI
3957,SmaI
3951,BamHI
3945,SpeI
3939,XbaI
3932,XmaIII
3931,NotI
3923,SacII
3920,BstXI
3915,EcoICRI
3915,SacI
BclI,370
SauI,420
TthI,527
NcoI,773
BalI,809
BspMII,1078
PvuII,1176
BbuII,1500
AatII,1567
Ksp63,1696
XmnI,1886
PvuI,2118
Eco31,2418
cam
amp
eae
PT7

FIGURE 6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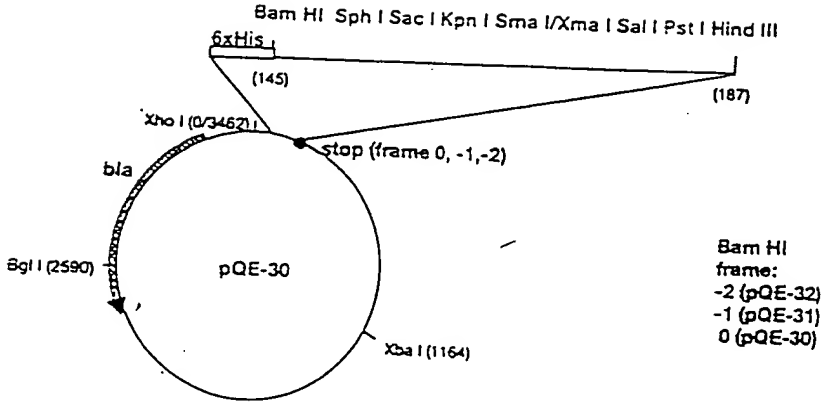
7161,Asp718
7155,ApaI
7146,XhoI
7146,SciI
7125,HindIII
7030,AflII
6586,NsiI
SauI,420
TthI,527
NcoI,773
BalI,809
BspMII,1078
PvuII,1176
cam
AatII,1567
pEB310
7382 bps
eae
XmnI,1886
amp
5154,EcoRV
4972,NspHI
4972,AflIII
4774,StuI
4749,BstEII
PT7
4045,NdeI
3957,XmaI
3957,SmaI
3951,BamHI
3945,SpeI
3939,XbaI
3932,XmaIII
3931,NotI
3920,BstXI
3915,EcoICRI
3915,SacI

FIGURE 7


Bam HI Sph I Sac I Kpn I Sma I/Xma I Sal I Pst I Hind III
6xHis
(145)
(187)
Xho I (0/3462)
stop (frame 0, -1,-2)
bla
Bgl I (2590)
pQE-30
Xba I (1164)
Bam HI
frame:
-2 (pQE-32)
-1 (pQE-31)
0 (pQE-30)

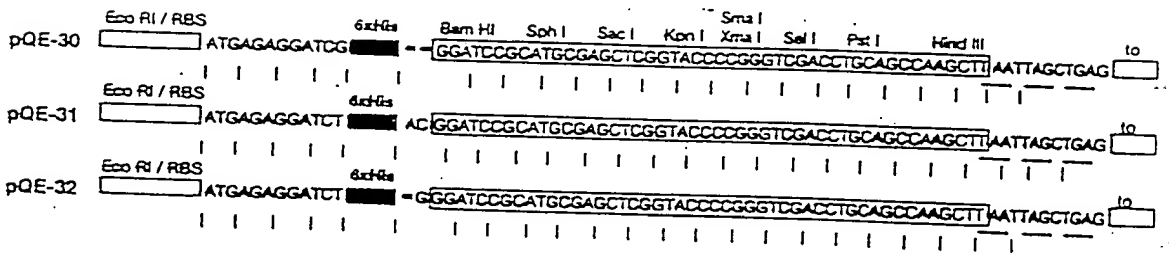
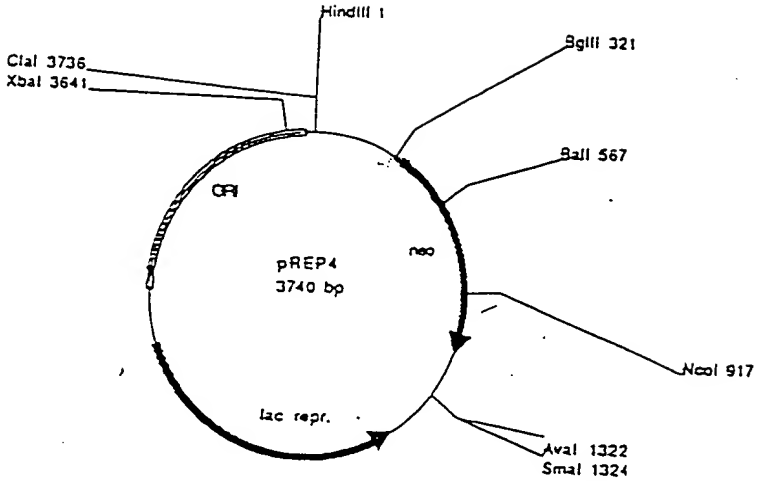

Eco RI / RBS
6xHis
Bam HI
Sph I
Sac I
Kpn I
Sma I
Xma I
Sal I
Pst I
Hind III
pQE-30
ATGAGAGGATCG
GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
to
pQE-31
ATGAGAGGATCT
AC GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
to
pQE-32
ATGAGAGGATCT
G GGATCCGCATGCGAGCTCGGTACCCCGGGTCGACCTGCAGCCAAGCTT
AATTAGCTGAG
to

FIGURE 8

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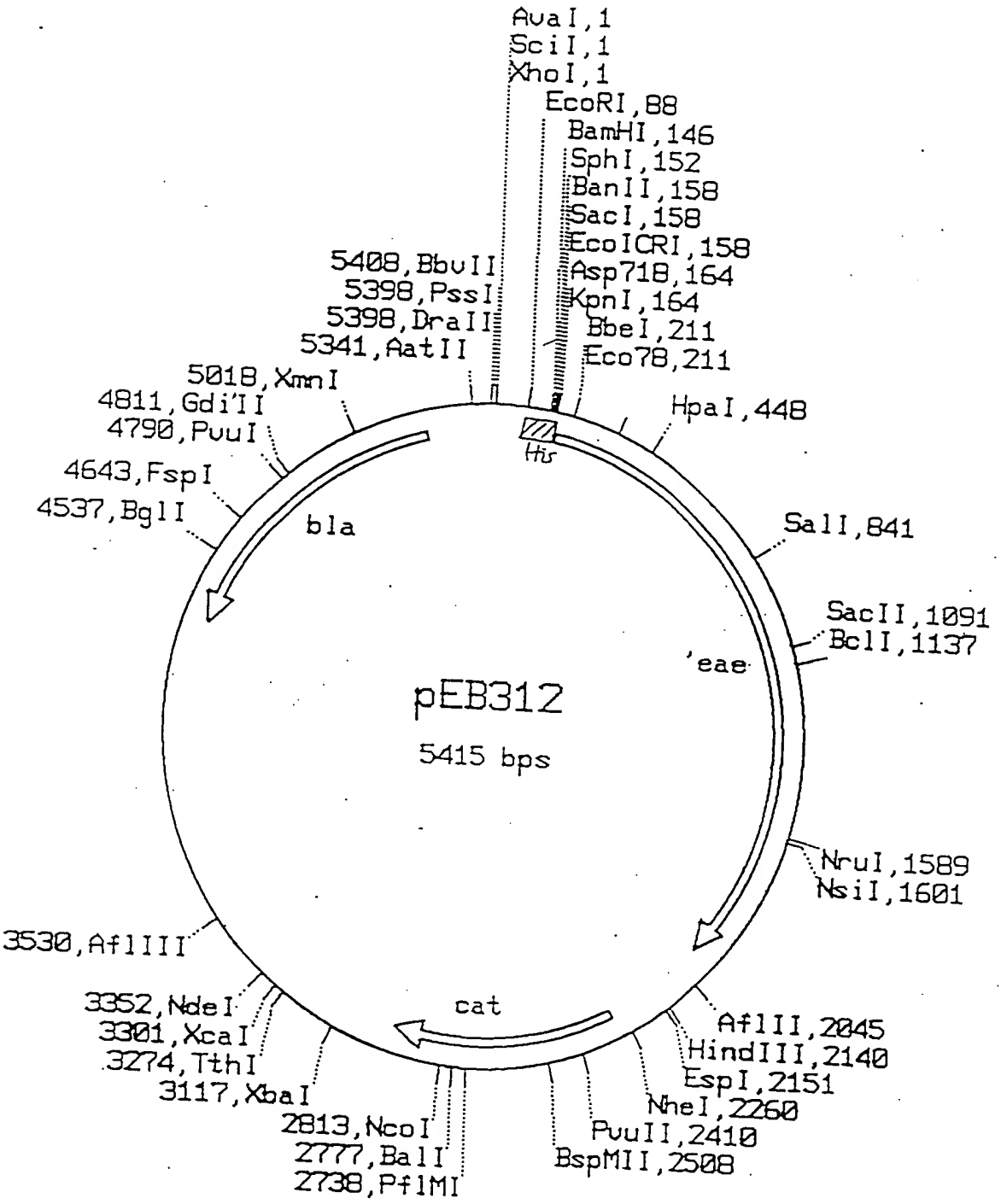
      Xho I                                                operator I
1     CTCGAGAAAT CATAAAAAAT TTATTTGCTT TGTGAGCGGA TAACAATTAT
                                                                TATA-Box
                operator II                    Eco RI
51    AATAGATTCA ATTGTGAGCG GATAACAATT TCACACAGAA TTCATTAAAG
           +1 start mRNA
                ATG →                          6xHis
101   AGGAGAAATT AACTATGAGA GGATCCGCATC ACCATCACCA TCACGGATCC
      RBS/SD                                                Bam HI

151   GCATGCGAGC TCGGTACCCC GGGTCGACCT GCAGCCAAGC TTAATTAGCT
        Sph I   Sac I   Kpn I   Sma I   Sal I   Pst I   Hind III
                                                        Stop 1 2 3

201   GAGCTTGGAC TCCTGTTGAT AGATCCAGTA ATGACCTCAG AACTCCATCT
```

FIGURE 9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XhoI,1
EcoRI,88
BamHI,146
SphI,152
BanII,158
SacI,158
EcoICRI,158
Asp718,164
KpnI,164
BbeI,211
Eco78,211
HpaI,448
SalI,841
SacII,1091
BclI,1137
NruI,1589
NsiI,1601
AflII,2045
HindIII,2140
EspI,2151
NheI,2260
PvuII,2410
BspMII,2508
2813,NcoI
2777,BalI
2738,Pf1MI
3352,NdeI
3301,XcaI
3274,TthI
3117,XbaI
3530,AflIII
5408,BbvII
5398,PssI
5398,DraII
5341,AatII
5018,XmnI
4811,GdiII
4790,PvuI
4643,FspI
4537,BglI
bla
cat
'eae
His
pEB312
5415 bps

FIGURE 10

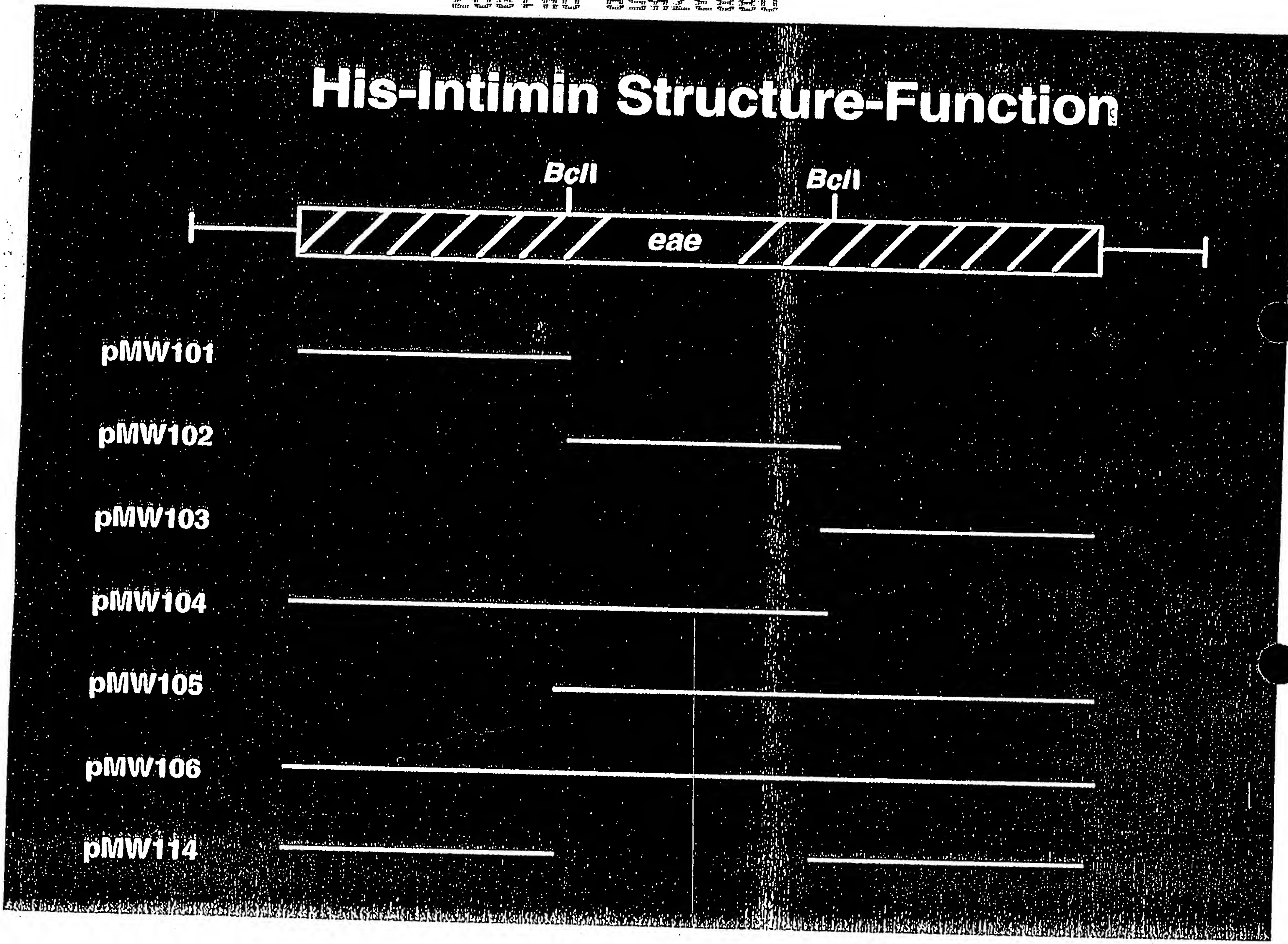
His-Intimin Structure-Function
BclI
BclI
eae
pMW101
pMW102
pMW103
pMW104
pMW105
pMW106
pMW114

FIGURE 11

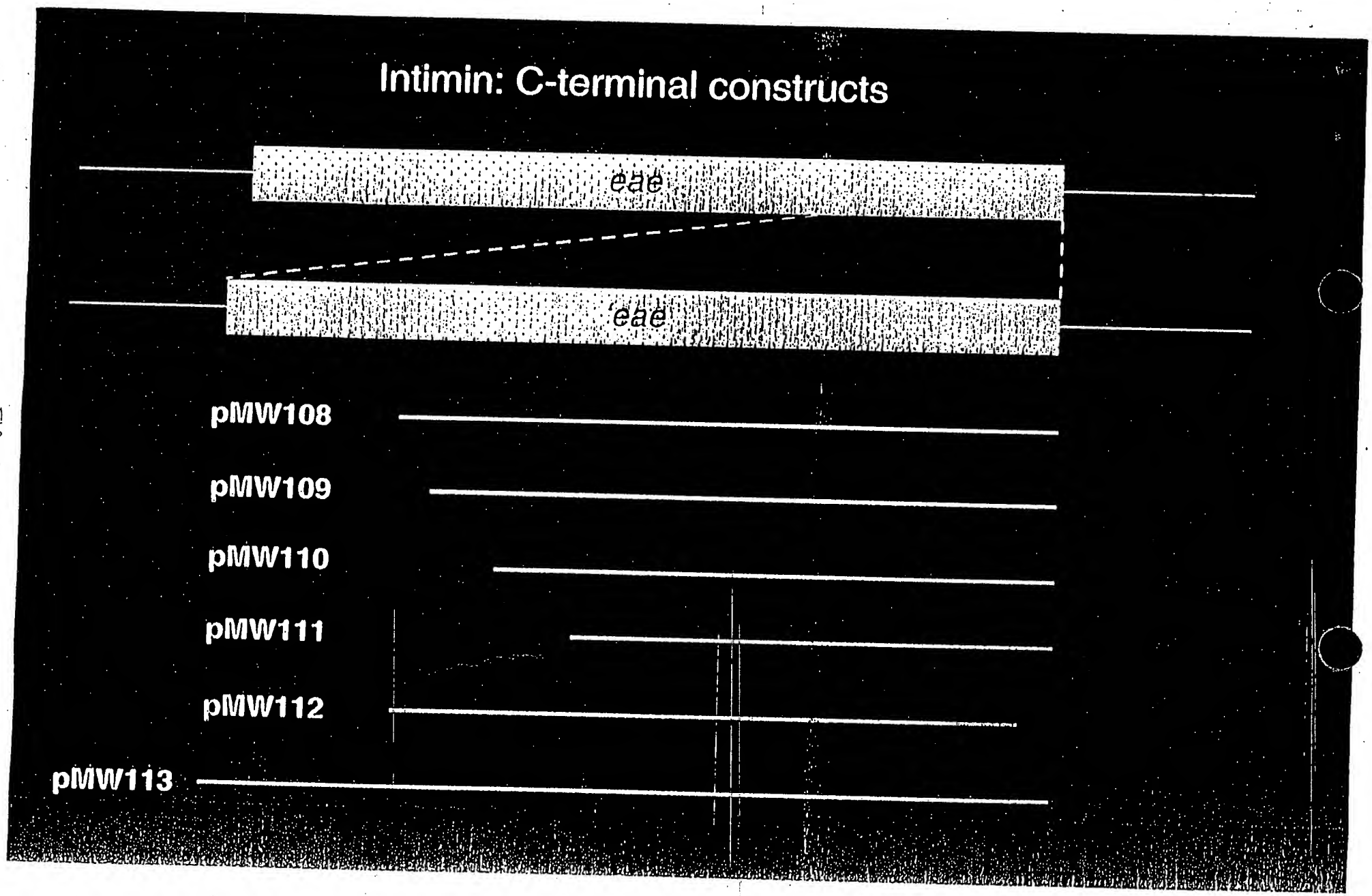

FIGURE 12

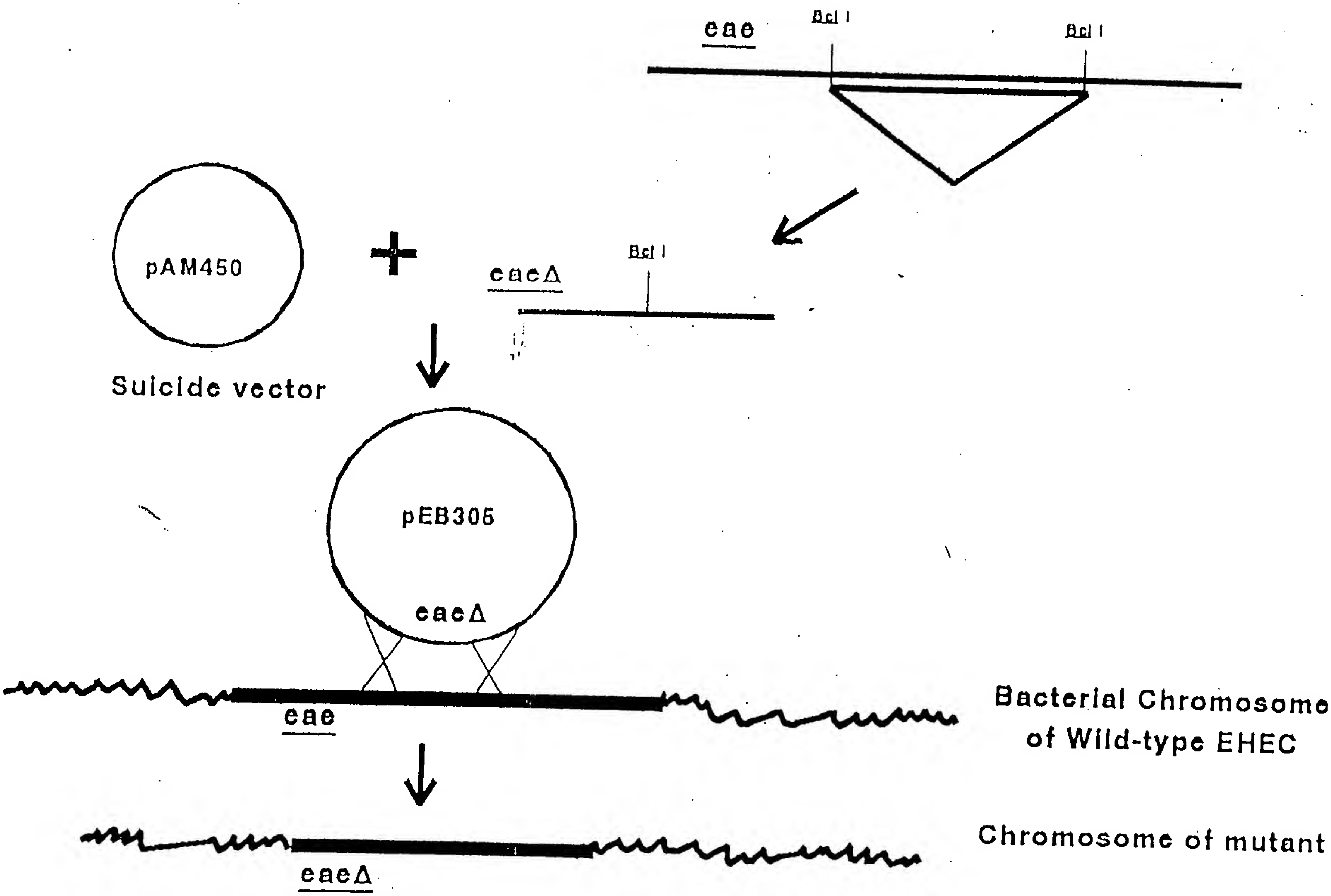
eae
Bcl I
Bcl I
pAM450
Suicide vector
caeΔ
Bcl I
pEB305
caeΔ
eae
Bacterial Chromosome
of Wild-type EHEC
eaeΔ
Chromosome of mutant

FIGURE 13

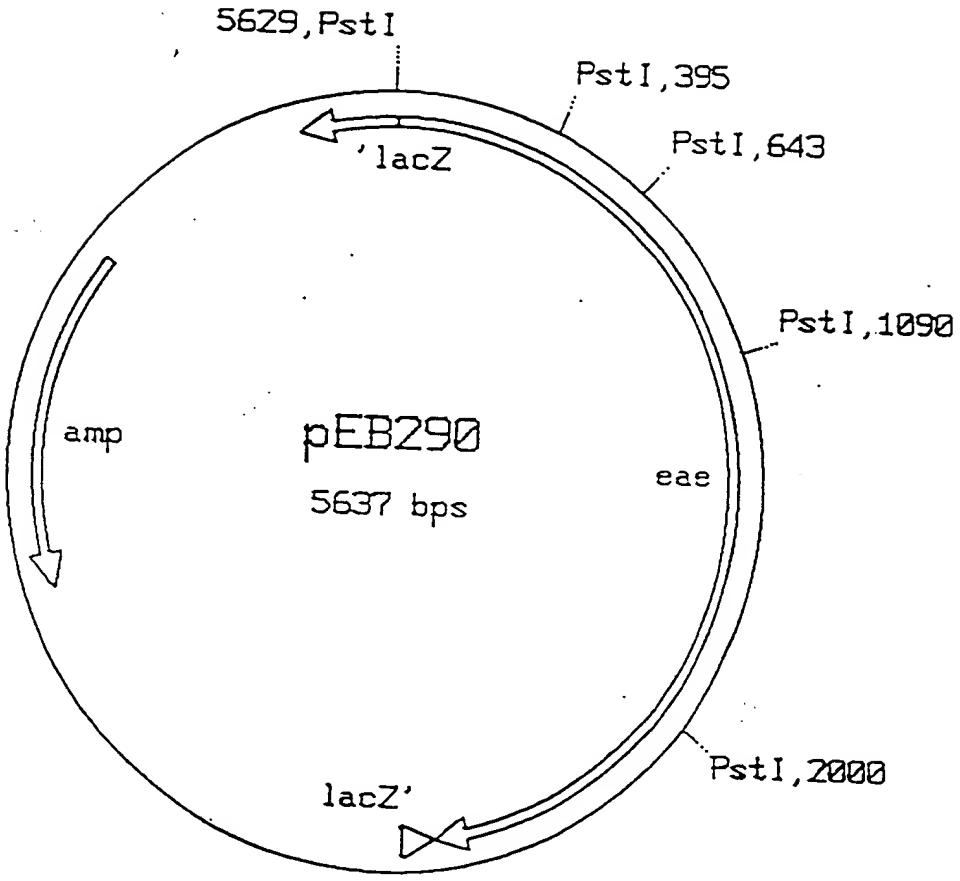

FIGURE 14

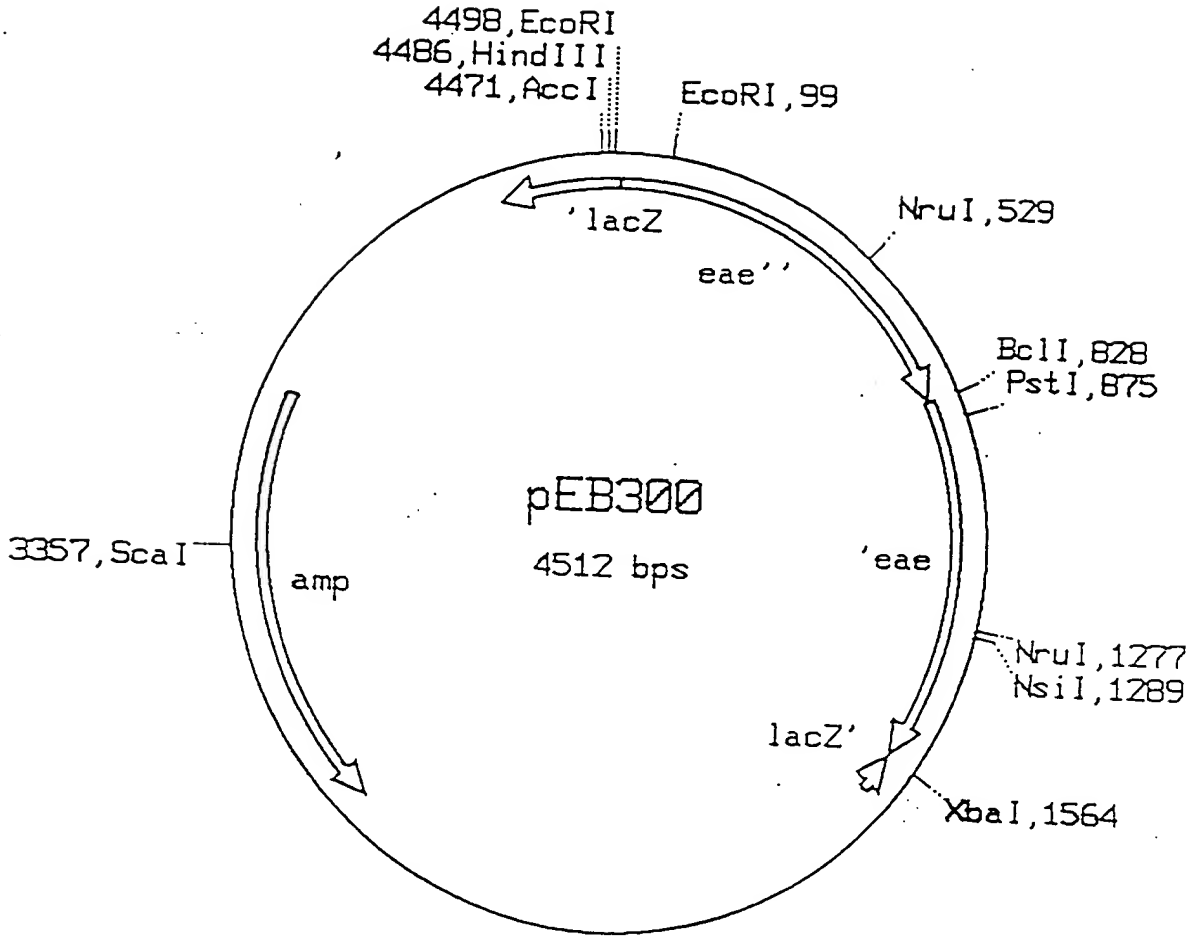
4498,EcoRI
4486,HindIII
4471,AccI
EcoRI,99
'lacZ
eae''
NruI,529
BclI,828
PstI,875
pEB300
4512 bps
3357,ScaI
amp
'eae
NruI,1277
NsiI,1289
lacZ'
XbaI,1564

FIGURE 15

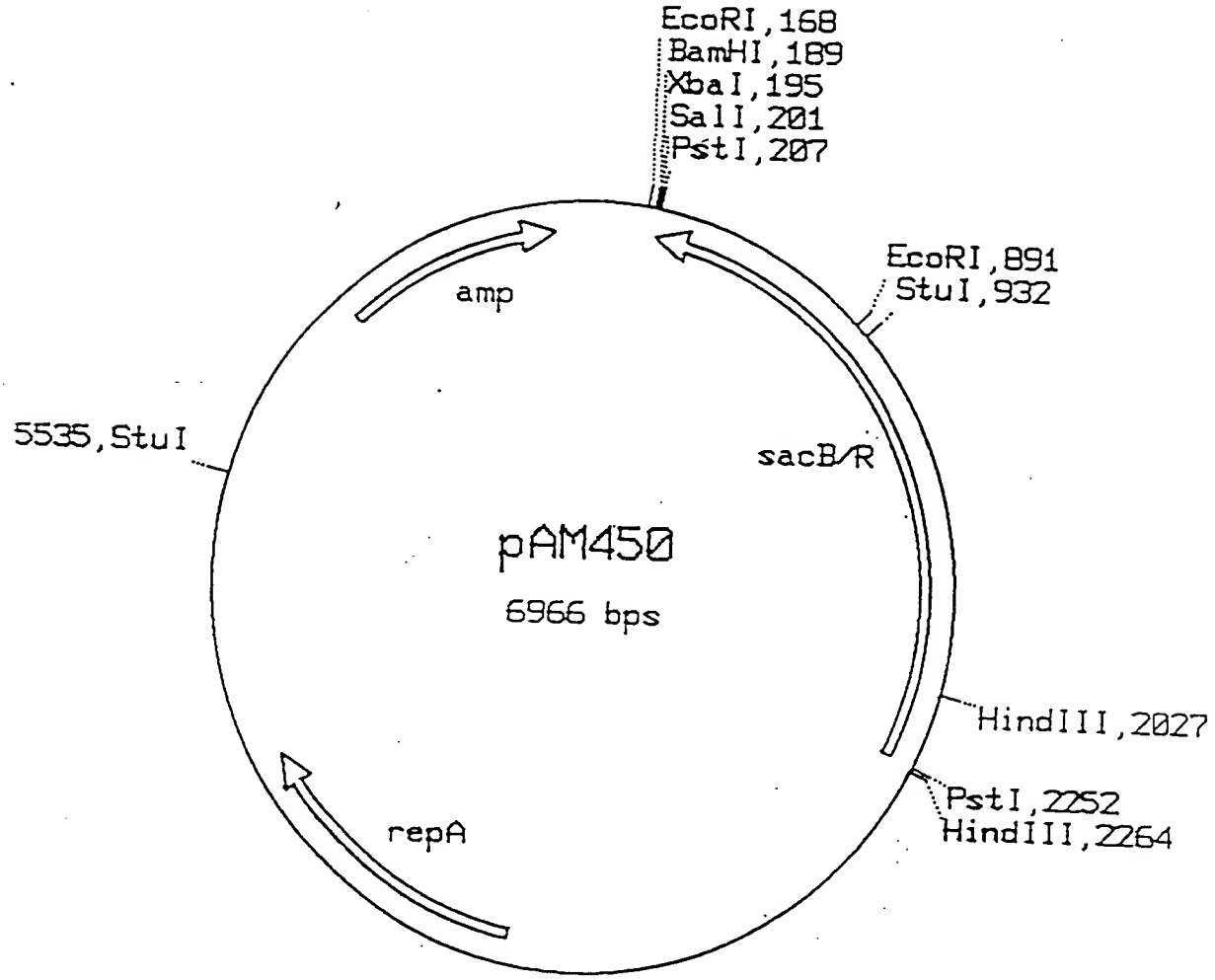
EcoRI,168
BamHI,189
XbaI,195
SalI,201
PstI,207
EcoRI,891
StuI,932
amp
5535,StuI
sacB/R
pAM450
6966 bps
HindIII,2027
PstI,2252
HindIII,2264
repA

FIGURE 16

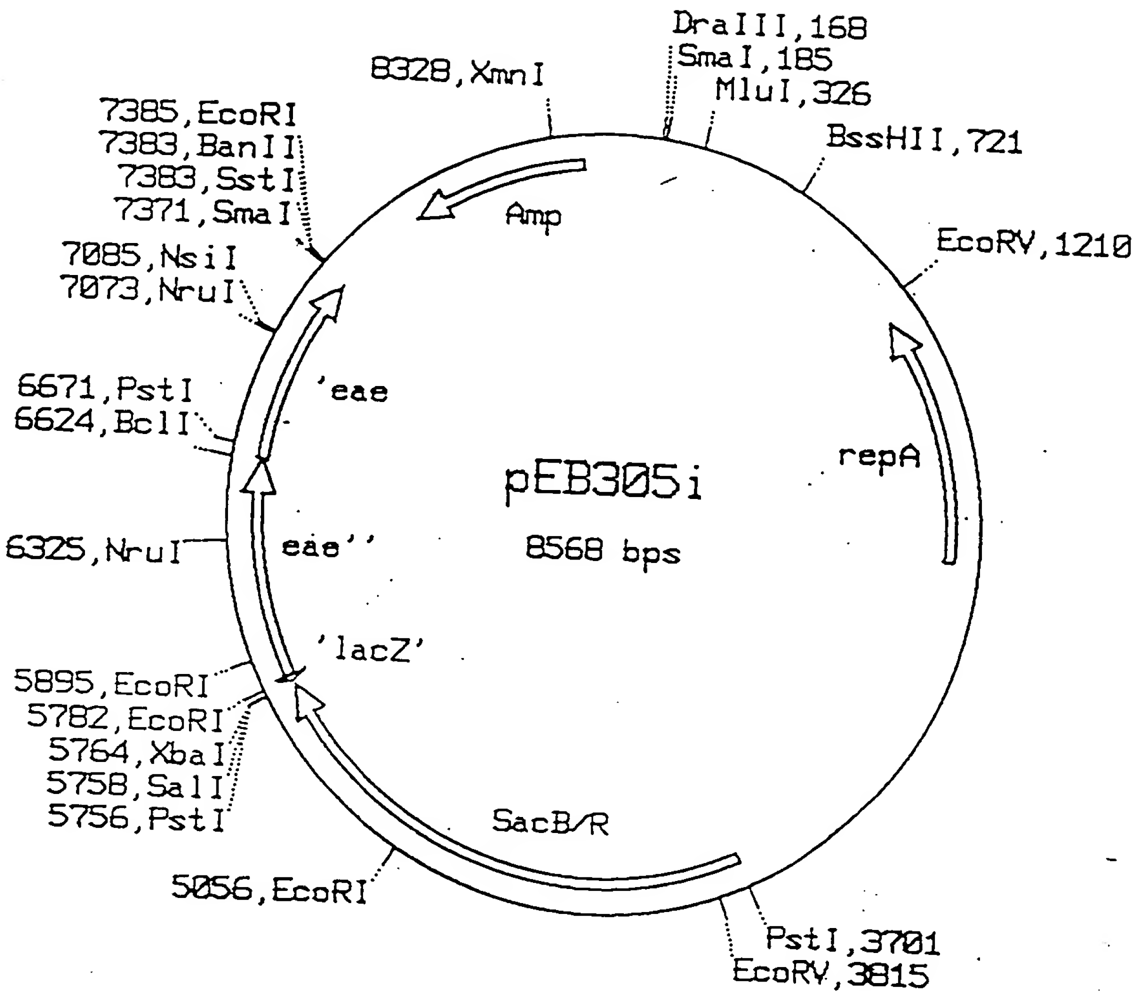

FIGURE 17

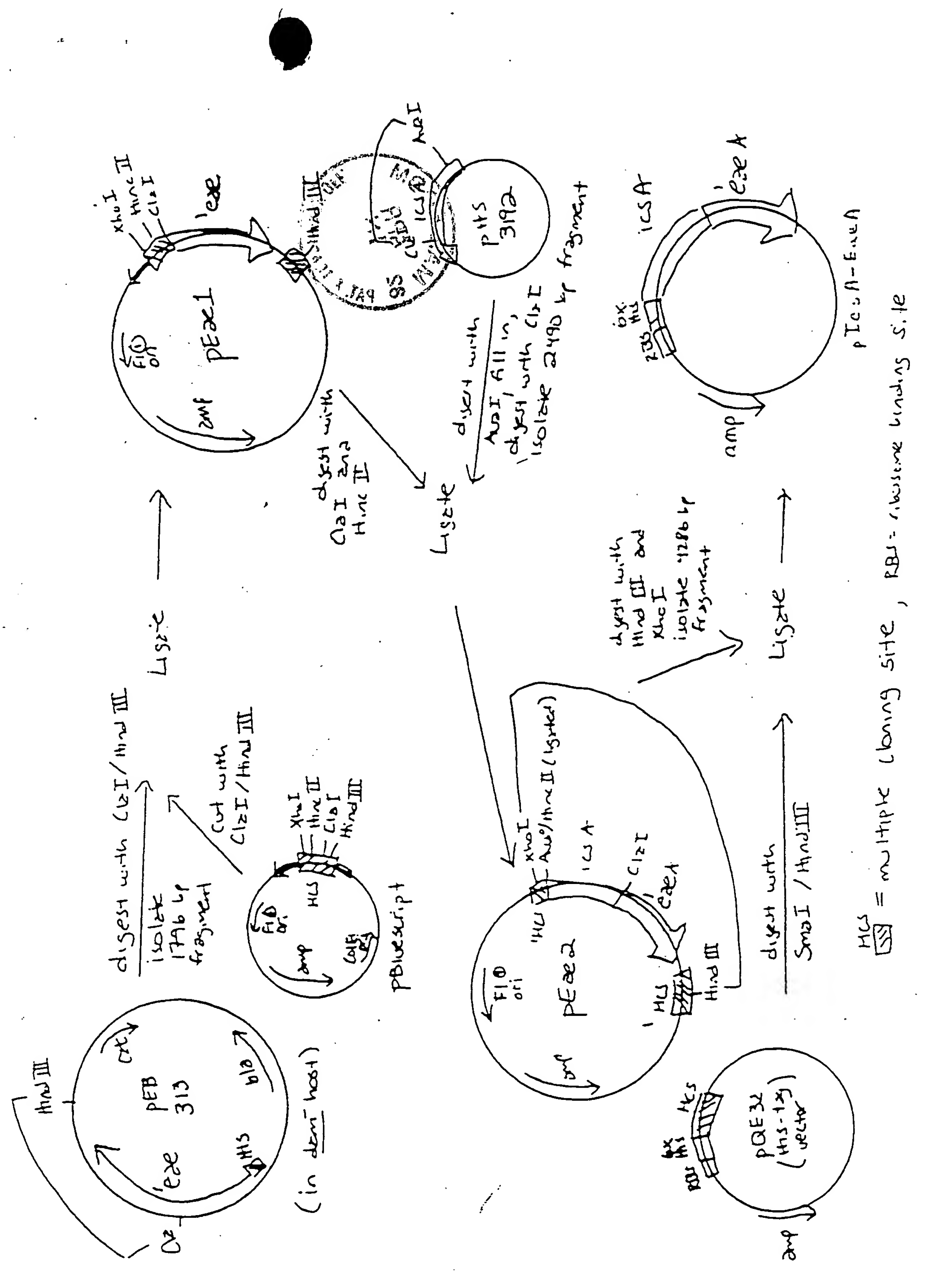
pEB 313
(in dam⁻ host)
pBluescript
pEae1
pEae2
pQE32 (His-tag vector)
Ligate
Ligate
Ligate
= multiple cloning site, RBS = ribosome binding site

FIGURE 18